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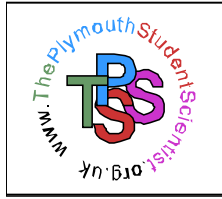
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# An investigation into the cardiac implications of bradycardia in marine sports practitioners

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## Abstract

This study investigated the effect of the mammalian diving reflex (MDR) on the R-R intervals, mean heart rate (MHR) and mean arterial blood pressure (MABP) on 40 healthy subjects. Two groups were compared a group of SCUBA divers and a group of non SCUBA divers. Each group consisted of ten males and ten females. Subjects completed seven protocols: three in air; breathing (BA); apnea (AA); breathing through a snorkel (BSA) and three facially immersed; breathing through a snorkel ( $23\pm1^{\circ}\text{C}$ ) (BSI); apnea ( $35\pm1^{\circ}\text{C}$ ) (AWW); apnea ( $23\pm1^{\circ}\text{C}$ ) (AWR) apnea ( $10\pm1^{\circ}\text{C}$ ) (AWC). A negative correlation was observed between diving experience and bradycardia. A positive correlation was observed between MABP and R-R intervals with respect to diving experience. No relationship was observed between the MHR, MABP or the R-R intervals in either the male or female subject groups. However, there was significant variance between the MHR, MABP and R-R complex intervals of divers and non-divers ( $P \leq 0.0085$ ). Significant variance was observed between male scuba divers, female scuba divers, male non-scuba divers and female non-scuba divers in MHR, MABP and R-R interval ( $P \leq 0.0085$ ). The results of this study suggest that the mammalian diving reflex is exacerbated in humans by repeat exposure to apnea with immersion. It also suggests that males and females are physiologically similar when considering MDR.

**Keywords** Apnea · Diving Reflex · Bradycardia · Cardiac Response

## **Introduction**

The mammalian diving response has been widely observed and documented in diving birds, mammals including dogs (Hermes-Lima and Zenteno-Savin, 2002), (Butler and Jones 1997) and in humans (Hong. 1989). The diving response is qualitatively similar across species in that a combination of bradycardia and vasoconstriction in the extremities along with stimulation of the cold facial receptors facilitates oxygen conservation (Daly 1997), (Elsner & Gooden 1983). Uniquely in humans, after immersion, an initial phase of tachycardia ( $10 \pm 2$  seconds) which has been associated with the breathhold-without-immersion condition (Hurwitz and Furedy 1986) is followed by a second phase of bradycardia which lowers mean heart rate (MHR) by 21-33% (Andersson et al 2002) which is usually accompanied by T-wave attenuation (Hurwitz and Furedy. 1986). After the initial sympathetic heart rate acceleration a vagally controlled deceleration occurs which occurs in parallel to sympathetic activity which continues to attenuate the T-wave amplitude (Levy. 1997). Bradycardia is accompanied by an increase in mean arterial blood pressure (MABP) in the order of 34-42% (Anderson et al 2002). In opposition to bradycardia stimulation of the facial chemoreceptor's and cold receptors by immersion causes vasoconstriction of the arterial vascular tree and subsequently increased central blood volume and blood pressure which then trigger sympathetic activity and therefore tachycardia through the brainbridge reflex which works to attenuate bradycardia (Duprez et al 2000). Apnea alone has been observed to trigger the diving response however, it is far less pronounced than when combined with the stimulation of cold facial receptors by facial immersion in water at a temperature around 0°C (Elsner and Gordon 1983).

Therefore to initiate a pronounced diving response apnea needs to be accompanied by stimulation of the facial cold receptors and the chemoreceptor's of the subject, by facial immersion to the temporal bone in water of a temperature between 10–15 °C (Daly 1997).

### **Heart Rate and R-R interval Data Collection**

Kingsley et al (2005), conducted a comparative study into the differences between Reynolds digital ambulatory clinical electrocardiogram (ECG) and the polar 810s heart rate monitor for recording the length of consecutive cardiac periods (R-R intervals) and heart rate. No significant differences were found between the two devices for heart rate ( $\pm 2$  beats) and R-R interval data ( $\pm 10$ ms) at any intensity. However, despite similar frequencies being attained during rest, Kingsley et al (2005) found significant differences between the ECG frequency ranges during moderate to intensive exercise.

### **Heart Rate Variability and Bradycardia**

Heart rate variability is a measure of the beat to beat variation in the MHR. The R-R interval is a measure of the time duration between one ventricular contraction and the next (Hampton, 1998). A time series can be analysed using the mathematical transformation Fast Fourier Transform which converts the heart rate variability data into power spectral density which discerns the amount of variation at a specific frequency (Heartmath 1991). High frequency activity has been linked to the parasympathetic nervous system and primarily respiration. Low frequency activity has been linked to a combination of parasympathetic and sympathetic nervous activity and stimulation of the baroreceptors in the arterial ventricular network (Heartmath 1991).

There have been comparatively few studies investigating the relationship between MDR and electrical activity across the heart and in particular heart rate variability. Bonneau et al (1989), studied electrocardiogram (EKG) traces for 10 divers (9 males, 1 female) of varying experience, over a minimum of three consecutive apnea dives to depths of 6, 9, 12 and 15 metres in a 15m pool at 28°C. A negative correlation was found between diving experience and bradycardia. Six of the divers experienced shallow water cardiac arrhythmias some of which were bigeminal (a double pulse beat). Some arrhythmias manifested as isolated occurrences but often occurred in multiples. Bonneau et al (1989) surmised that the arrhythmias were associated with forced expiration through the snorkel at the end of the dive. Lafay et al., (1995) studied ECG traces for three elite divers during a 71 atmosphere saturation dive. All three divers experienced marked bradycardia during the compression phase of the dive which subsequently levelled off. The ECG traces also showed similar characteristics between all three divers with little to no significant change in the P-R, QRS or Q-T intervals. However, a rightward shift was observed between the P and T vector angles. Lafay et al (1995) concluded that the rightward shifts correlated with time and gas density respectively, believing that a marked increase in interthoracic pressure was due to dense gas breathing which in turn prevented myocardial repolarisation. Therefore, the researchers suggested that micro bubbles within the cardiac tissue were affecting the impedance and suppressing the amplitude of the ECG complex. The divers in this study continuously breathed a helium oxygen mix.

Heyashi et al (1997) studied the effect of apnea and controlled breathing, with and without facial immersion on vagal activity and heart rate variability. 12 trials were performed on 15 subjects, controlled breathing at 5, 10, 15, 20, 30 breathes per

minute<sup>-1</sup> and uncontrolled breathing with and without facial immersion. During immersion tests subjects breathed through a short snorkel in 9°C (±1°C) water. Heyashi et al (1997) observed significant increases in SD(RR) and CV(RR) ( $P \leq 0.05$ ) with facial immersion and an increase in R-R mean at 20 breathes per minute<sup>-1</sup> ( $P \leq 0.05$ ). No change was observed in oxygen uptake, tidal volume, end-tidal O<sub>2</sub> and CO<sub>2</sub> partial pressure. Heyashi et al (1997) concluded that immersion alone was sufficient to increase heart rate variability; therefore facial immersion alone increases vagal activity.

Kinoshita et al (2006) studied the effect of facial immersion and apnea on heart rate variability in 8 healthy, non-smoking subjects, 4 male and 4 female subjects participated in the study. The subject's completed 6 protocols 4 with and 2 without apnea with and without facial immersion and in warm and cold water. Kinoshita et al (2006) found significant increase in R-R intervals between the breathing through a snorkel without immersion condition and the apnea with immersion in cold water condition. No significant difference was found between any of the other conditions and no increase in either systolic or diastolic blood pressure was observed. Kinoshita et al (2006) agreed with Heyashi et al (1997) that Immersion without apnea was responsible for increased vagal activity and therefore bradycardia. However, they added that due to limitations in the time-delay indices it was impossible to separate out sympathetic and parasympathetic nervous activity and therefore determine definitively the primary cause of the interval change.

The effects of ambient air temperature and water temperature on heart rate during the manifestation of MDR were investigated by Schagaty et al (1996). 23 subjects were exposed to three 60 minute exposures at 10, 20 and 30°C air temperatures and facial

immersion at 10, 20 and 30°C with apnea. Schagaty et al (1996) recorded oral and skin temperatures. A positive correlation was observed between ambient air temperature and bradycardia and a negative correlation between water temperature and bradycardia. Schagaty et al (1996) concluded that human bradycardia resulting from facial immersion with apnea was inversely proportional to water temperature within a range determined by ambient air temperature. Therefore the difference between the ambient air temperature and the water temperature is of primary importance rather than either the air or water temperatures in isolation.

## **Existing Protocols**

The following protocol was suggested by Hiebert & Burch (2003) in their review of laboratory test procedures for testing the human diving response. Hiebert & Burch (2003) on the observations of Daly (1997) suggested that a bowl filled with fresh water at a temperature of 12.5°C ( $\pm 2.5$  °C) be used for facial immersion. To elicit a maximal response, the subject takes a deep, sub-maximal breath (without hyperventilation), and immerses their face in the water to the temporal lobes (Schuitema & Holm 1988; Daly, 1997). For maximal results, a minimal test duration of 30 seconds should be used (Hiebert & Burch 2003) though the subject should be aware that they can voluntarily terminate the test at any point. In the protocol used by Hiebert & Burch (2003) two spotters were used one to aid the subject during the experiment and collect radial pulse data using a heart rate monitor and the other to control the duration of the test using a stopwatch. At the end of each test the subject was allowed a rest period of 10 minutes to allow for metabolisation of lactate produced due to apnea and therefore anaerobic respiration during the experiment.

To facilitate comparisons between different stress conditions, paired protocols were used, which in turn allowed the testers to discern which stimulus was responsible for each physiological aspect of the diving response (Hiebert and Burch, 2003). The protocol was then extended to investigate the effect of temperature on the diving response. Ice packs were used, rather than immersion in water to determine the extent to which stimulation of the cold receptors rather than immersion triggered the response (Hiebert and Burch 2003). To limit experimental errors due to variations in basal rate throughout the day and habituation in the subjects Hiebert & Burch (2003) suggested that the order in which the protocols are conducted should be randomised. In their experiments the data was first analysed using an ANOVA (analysis of variance) test prior to paired t-tests.

To date many studies have been conducted into the causal factors for instigating MDR and the effects of these causal factors on the MHR and MABP of subjects undergoing the response. However, as far as the researcher can ascertain very little research has been conducted to investigate the heart rate variation of individuals during MDR and that which has been conducted has involved comparatively small sample groups. Therefore this study will aim to investigate the effects of MDR on heart rate variation and in so doing increase the level of understanding in this area.

## **Methods**

### **Subjects**

40 healthy subjects participated in the study twenty males and twenty females. The mean height, weight, age, resting heart rate and mean arterial blood pressure of the male group was  $1.78\text{m} \pm 0.20$ ,  $77\text{kg} \pm 20$ ,  $21 \text{ years} \pm 3$ ,  $65 \text{ bpm} \pm 15$ ,  $104 \text{ mmHg} \pm 15$



respectively. The mean height, weight, age, resting heart rate and mean arterial blood pressure of the female group was  $1.68\text{m} \pm 0.20$ ,  $61.9\text{kg} \pm 5$ ,  $22 \text{ years} \pm 4$ ,  $69\text{bpm} \pm 7$ ,  $104\text{mmHg} \pm 12$  respectively. The SCUBA diving group comprised of individuals who had a minimum of forty documented dives and a PADI open water qualification or equivalence. Testing took place over two weeks in January in England; the mean air temperature in the laboratory was  $\sim 23^{\circ}\text{C}$ . In accordance with institutional ethical approval all subjects provided written consent to participate. Subjects were asked not to alter their dietary habits or consume alcohol or beverages containing caffeine and to drink plenty of fluids and inform the tester of any prescribed medication prior to testing. Subjects completed a screening questionnaire and provided verbal confirmation that they had adhered to these instructions on arrival.

### **Experimental design**

The study was split into three phases completed in order. The first phase comprised of physical examination and anthropometric measurements of height, weight, lean mass, fat mass, percentage water, resting heart rate, blood pressure and R-R intervals.

Phase two was to familiarize the subjects with the protocol of the experiment. Subjects completed forty second breath holds both with and without facial immersion in water ( $23 \pm 1^{\circ}\text{C}$ ).

Phase three comprised of the seven experimental protocols completed in a randomised order.

## **MDR Protocol**

All subjects were given a fifteen minute preparation period prior to testing where they were asked to lay prone in silence. Testing occurred in a randomised order to limit habituation, with a five minute interval between each test. For non-immersion tests the subject stood erect and relaxed. For the immersion tests the subject leant forward at the waist over the laboratory bench with elbows resting on the lab bench to support them and head down. Each test lasted for 40 seconds, timed by a stopwatch. Heart rate and blood pressure were taken in the last ten seconds of the procedure using a heart rate monitor, sphygmomanometer (A.C. Cassor & son (surgical) LTD, Essex, England) and stethoscope (3 M Littmann Classic 2 SE, St Paul, Minnesota). The R-R interval was recorded onto a s810i Polar heart rate monitor (Polar electro, OY, Finland). The data was then transferred into a spreadsheet via the polar precision software (Version 3.01) for analysis. For all tests involving apnea subjects took a deep sub maximal breath and held it for the duration of the 40 second test. Subjects were dissuaded from breathing-up or hyperventilating during the test. Test subjects were told to immerse their face up to the temporal bone during immersion tests. The temperature of the water was measured using a digital thermometer (Comark N9092, Stevenage, England) and set at the desired temperature prior to each test. All equipment was disinfected between tests using 2% hydrochloride solution.

## **Heart Rate Data Collection**

As the study was conducted under low exertion conditions, in accordance with the findings on Kingsley et al (2005), a Polar Heart rate monitor 810s was used to collect the R-R interval and heart rate data. After collection the data was uploaded to a spreadsheet for analysis.

## **Heart Rate Variation Analysis**

The heart rate variation data was transformed and analysed using heart rate variation analysis software V1.1 from the Biomedical Signal Analysis Group. As the data ascertained during this experiment may be different from values based on the general population frequency values derived from the non-parametric model were used.

## **Statistical analysis**

All of the data was plotted to determine normality prior to statistical testing.

The Heart rate, blood pressure and R-R intervals for the subject groups and the seven protocols were all analysed using analysis of variance (ANOVA) prior to paired t-tests to assess variance in the means. Three Bonferroni adjustments were used to limit false significance due to repeated t-tests.

Comparison of the mean heart rates, mean arterial blood pressures and R-R intervals of; male SCUBA divers, female SCUBA divers, male non-SCUBA divers, female non-SCUBA divers; 6 tests,  $0.05/6 = 0.0083$ . Alpha was set at  $P \leq 0.0083$ .

Comparison of the mean heart rates, mean arterial blood pressures and R-R intervals for the protocols; BA, AA, BSA, BSW, AWW, AWR, AWC; 21 tests,  $0.05/21 = 0.002$ .

Alpha was set as  $P \leq 0.002$ .

Comparisons between the non-parametric percentage powers from the R-R interval data for the protocols; BA, AA, BSA, BSW, AWW, AWR, AWC; 21 tests,

$0.05/21 = 0.002$ . Alpha was set as  $P \leq 0.002$ .

## Results

Tachycardia was observed in the first 10 ( $\pm 2$ ) seconds of the protocols AA, AWW, AWR, AWC followed by bradycardia which resulted in an overall decrease in mean heart rate from the initial rest state BA.

The study found no significant variance between the mean heart rate, mean arterial blood pressure and R-R intervals of males and females ( $P \geq 0.0085$ )

A negative correlation was observed between diving experience and bradycardia.

Bradycardia was more pronounced amongst the scuba diving group, with a 27% decrease in mean heart rate from the rest condition BA, to the full stress condition AWC (Fig. 1a). The non-scuba diving group also experienced bradycardia however, it was far less pronounced at 13%. No significant variance was observed between the mean heart rates of males and females during the experiment. However, a significant variance was observed between the mean heart rates of male and female scuba divers, male scuba and male non-scuba divers, male scuba and female non-scuba divers, female scuba and male non-scuba divers, female scuba and female non-scuba divers and male non-scuba and female non-scuba divers (Fig. 1b).

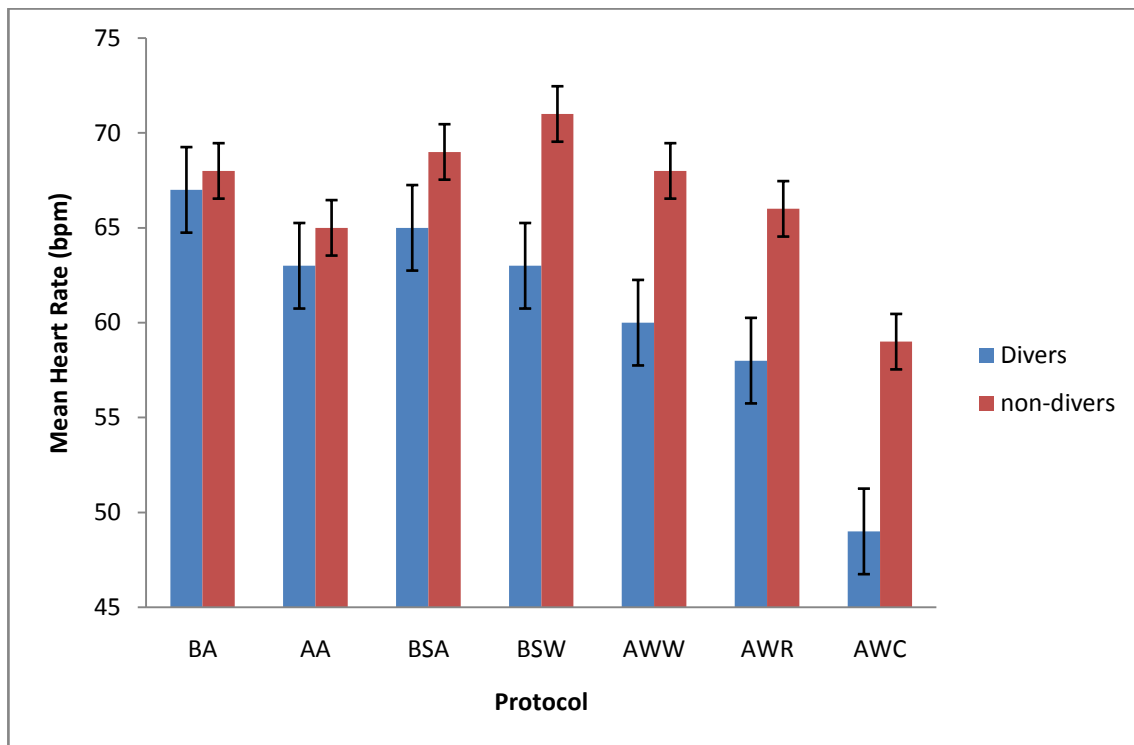


Figure 1a Mean Heart Rate (bpm) plotted against Protocol for divers and non-divers.

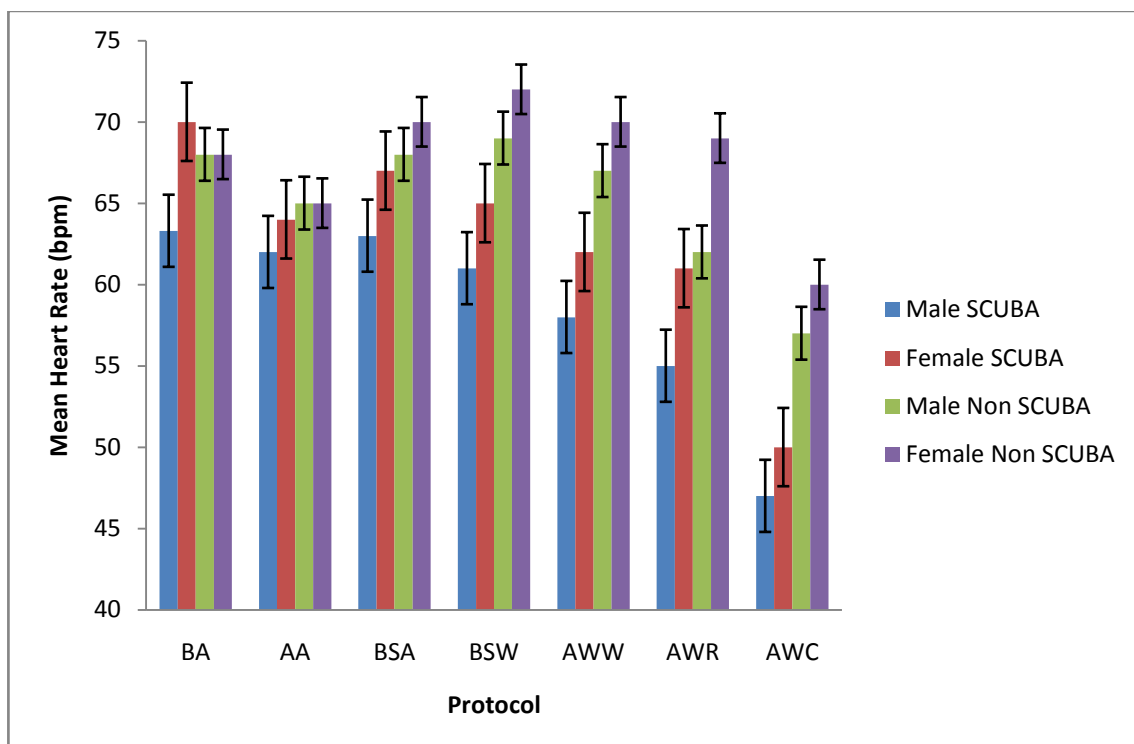


Figure 1b Mean Heart Rate plotted against Protocol for all subject groups

The study found that apnea alone was sufficient to cause a significant decrease in mean heart rate ( $P \leq 0.002$ ). However, no significant increase in mean arterial blood pressure was observed from the BA to AA condition ( $P \leq 0.002$ ).

Apnea with immersion in 35°C and 23°C water was insufficient to cause significant bradycardia.

A positive correlation was observed between MABP and water temperature and also between MABP and diving experience.

An increase of 216% in the time duration was observed in the R-R intervals of scuba divers which proved significant when compared to the increase of 116% observed in the non-scuba divers (Fig. 2a). However, no difference was observed between the R-R intervals of males and females. The heart rate variation data showed a significant variance between the R-R intervals of male scuba and male non-scuba divers, male scuba and female non-scuba divers and female scuba and male non-scuba divers. However, no significant variance was observed between the R-R intervals of male scuba and female scuba divers and female scuba and female non scuba divers (Fig. 2b).

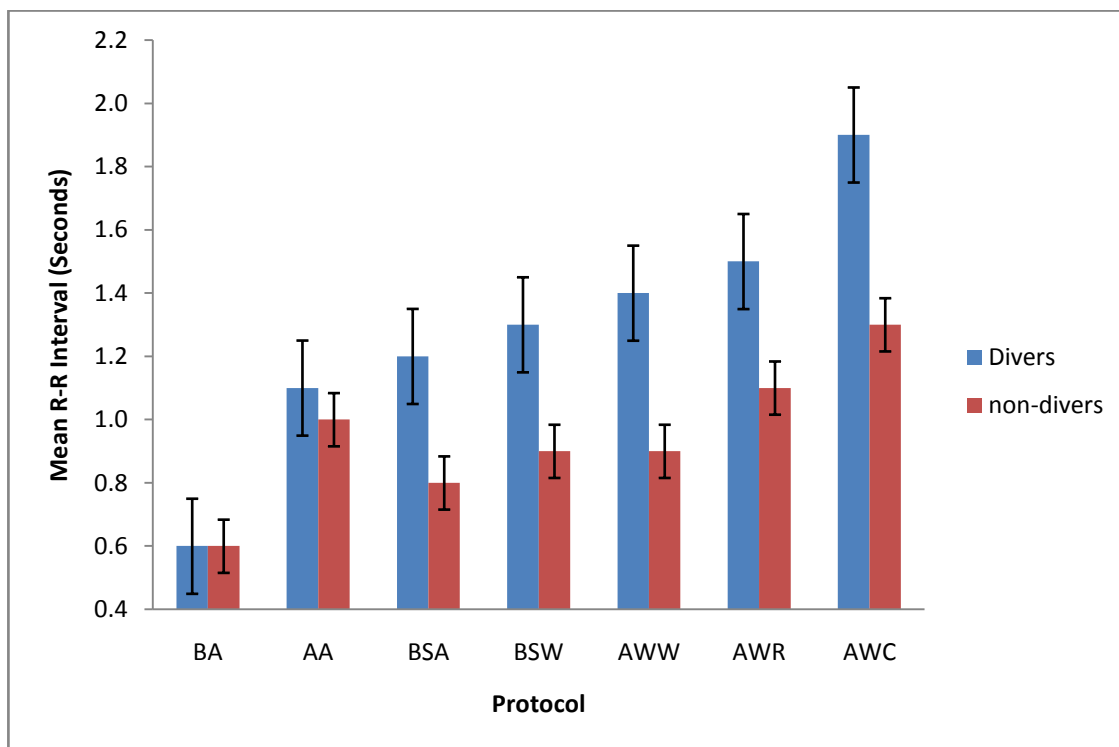


Figure 2a R-R interval in seconds plotted against Protocol for divers and non-divers.

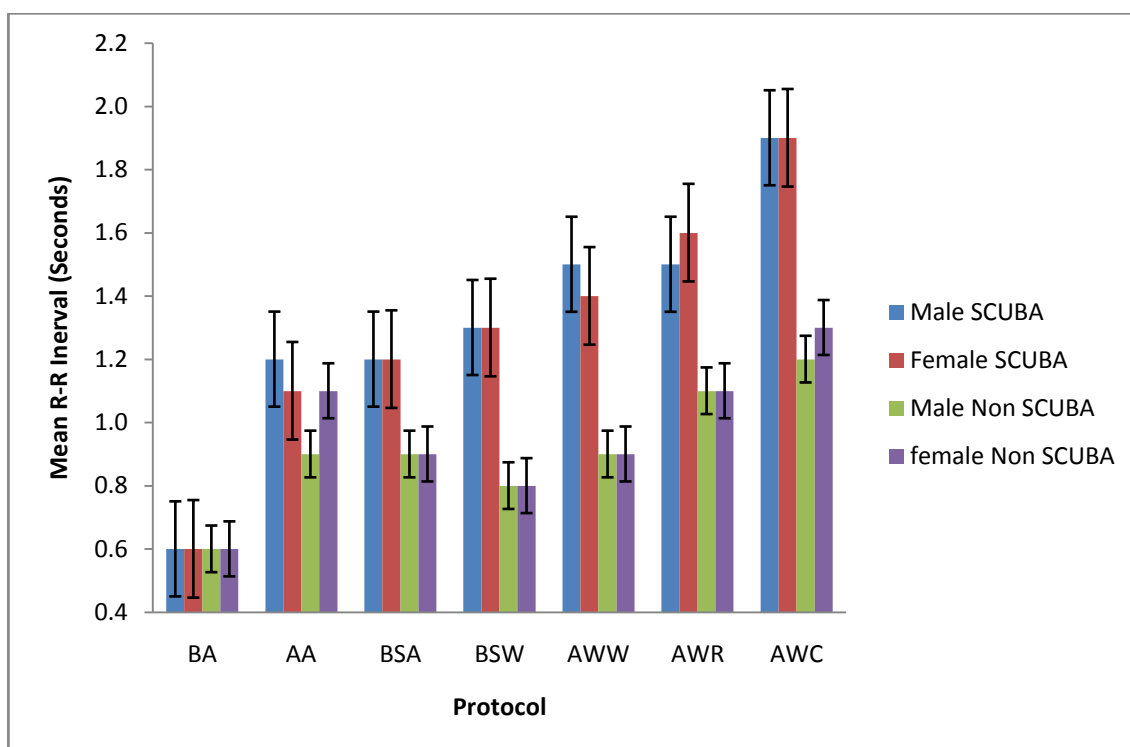


Figure 2b Mean R-R intervals in seconds plotted against Protocol for all groups.

A decrease in LF power and an increase in HF power were observed in all the subject groups from protocols BA to AWC (Fig 2c, Fig 2d). However, a significant variance was only observed between the LF and HF power of the BA, AA and AA to AWC conditions ( $\leq 0.002$ ).

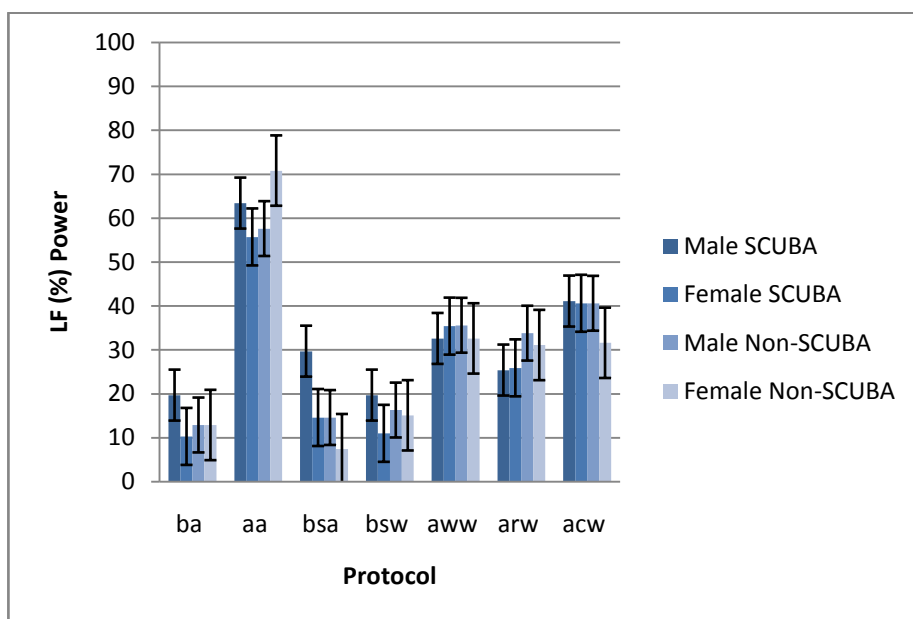


Figure 2c LF power plotted against Protocol for all Subject Groups.

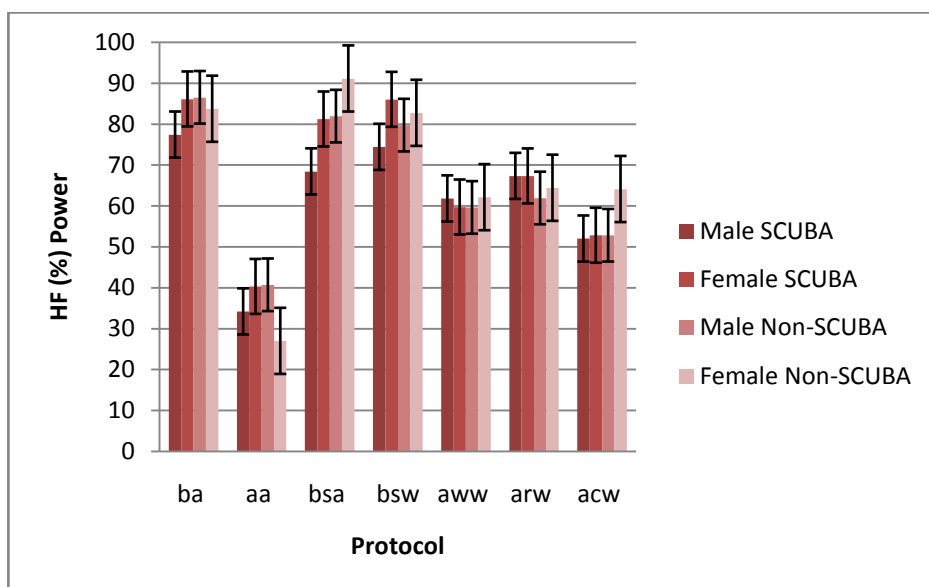


Figure 2d HF power plotted against Protocol for all Subject Groups.



Significant increases ( $\leq 0.002$ ) were observed in the LF/HF % power derived from the non-parametric values of all groups from the BA to AA and BA to AWC conditions (Fig 2e).

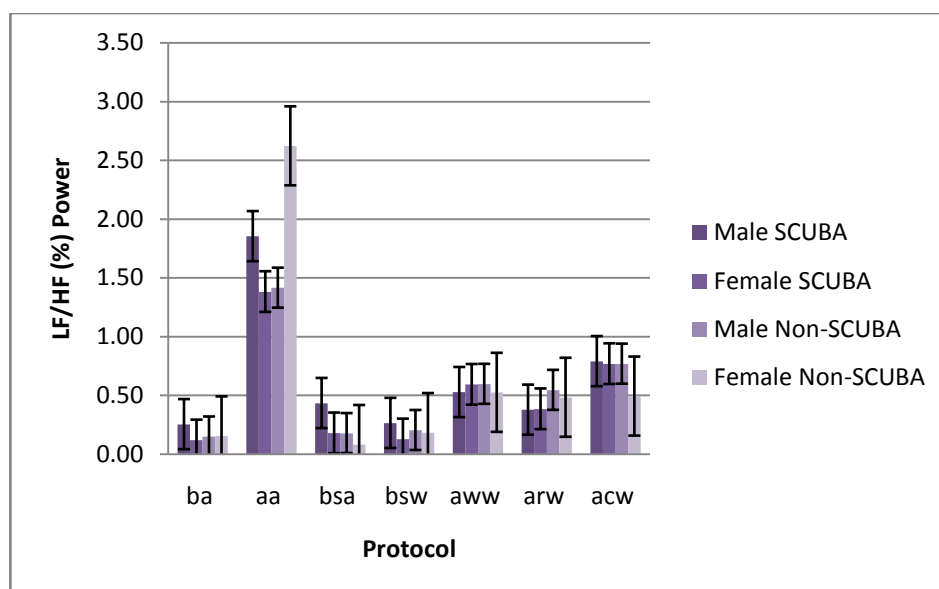


Figure 2e LF/HF (%) Power Plotted against Subject Group Derived from non-parametric analysis of the R-R interval Data for the Protocols BA, AA and AWC.

An increase in low frequency activity and a decrease in high frequency activity were observed in all the sample groups from the non-parametric spectral analysis across all the protocols from BA to AWC.

The mean arterial blood pressure of the diving group was significantly higher than that of the non-diving group (29%) (Fig. 3a). However, no variance was observed between the MABP data of males and females. A significant difference ( $P \leq 0.002$ ) was observed between the MABP of male scuba and male non-scuba divers, male scuba and female non-scuba divers and female scuba and female non-scuba divers. Though, no difference was observed between male scuba and female scuba divers and male scuba and female non-scuba divers.

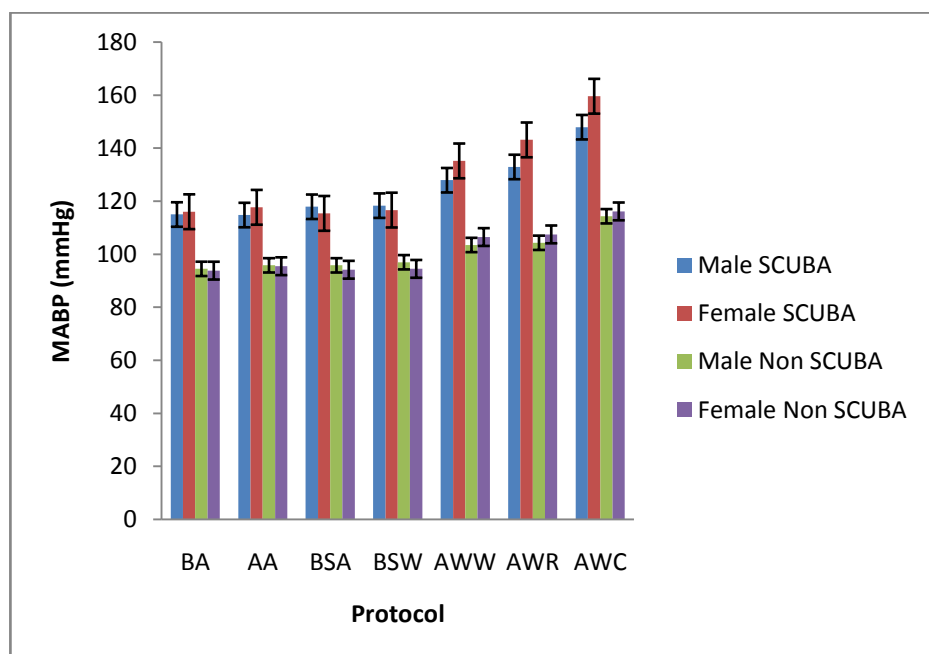


Figure 3a Mean Arterial Blood Pressure plotted against Protocol for all subjects.

## Discussion

The main finding of this study is that an apnea with immersion induced MDR, caused similar heart rate, blood pressure and R-R interval changes in both males and females. Therefore for the investigation of MDR, males and females are physiologically similar.

Tachycardia was observed in all the subject groups in the first 10 ( $\pm 2$ ) seconds followed by bradycardia leading to an overall decrease of 20% which concurs with Anderson et al (2002)

The mean heart rate data in this study concurs with Bonneau et al (1989) and shows a negative correlation between diving experience and bradycardia ( $P \leq 0.002$ ). However, the investigator didn't observe any cardiac arrhythmias in the test subjects though this may be due to the difference in experimental protocols.

The findings of this study concur with that of Kinishita et al (2006) and Hayashi et al (2006), that apnea alone is sufficient to trigger bradycardia (BA, AA) ( $P \leq 0.002$ )

Apnea in conjunction with immersion in 35°C and 23°C water (AWW, AWR) was insufficient to trigger bradycardia in any of the subject groups ( $P \leq 0.002$ ) However, apnea with immersion in all of the temperature conditions (AWW, AWR, AWC) caused significant hypertension ( $P \leq 0.002$ ).

Comparison between the AA condition and the AWC condition concurred with the findings of Daly (1997) in that stimulation of the facial cold receptors by temperatures between 10°C and 15°C in conjunction with stimulation of the chemoreceptor's by facial immersion causes a significantly greater increase in mean arterial blood pressure than apnea alone ( $P \leq 0.002$ ) This study also found a significant difference between the mean arterial blood pressures of subjects in the AWW, AWC and AWR, AWC conditions which may compound the fact that blood pressure changes during MDR are as a result of stimulation of the cold receptors and chemoreceptor's in the face.

This study found that apnea alone was insufficient to significantly raise mean arterial blood pressure in any of the subject groups (BA, AA) ( $P \leq 0.002$ )

The blood pressure data collected during this study indicates that repeated exposure to diving conditions including cold and increased ambient pressure increases sympathetic nervous activity which in turn increases an individual's ability to vasoconstrict the peripheral arterial ventricular tree which concurs with Asmussen and Kristiansson (1968).

Sinoarterial bradycardia was observed in the mean R-R intervals which proved significant between the BA and AA conditions ( $P \leq 0.002$ ) Significant variance was also observed between the R-R intervals of AA, AWR and AA, AWC ( $P \leq 0.002$ ) However, the variance between AA and AWW didn't prove significant in this study ( $P \leq 0.002$ ) These findings are notably different from Lafay et al., (1995) who found no significant change in the R-R intervals of their subjects despite observing notable bradycardia. However, it should be noted that the subjects in Lafay et al., (1995) were breathing a helium oxygen mix in a hyperbaric chamber. This study was conducted at atmospheric pressure on individuals experiencing apnea and facial immersion.

The data collected in this study suggests that R-R intervals are increased by immersion, primarily the stimulation of chemoreceptor's and cold receptors in the face and concurs with the findings of Heyashi et al (2006) and Kinoshita et al (2006). However, unlike Heyashi et al (2006) significant variance was observed between the BA and BSW, BA and AWW, and BA and AWR conditions as well as a significant increase in MABP.

The increase in the proportion of HF activity may indicate a greater proportion of parasympathetic nervous activity driven by the vagus nerve. However, further studies are needed to better understand the interdependent relationship between sympathetic and parasympathetic nervous activity during an elicited MDR response.

## **Conclusion**

In conclusion it has been shown that stimulation of the facial cold receptors and chemoreceptor's increases parasympathetic vagal activity and therefore causes bradycardia and an increase in R-R intervals. Apnea has been shown to cause an increase in MABP. No difference between male and female R-R intervals, HR and MABP was observed during this study. However, significant variances between divers and non-divers indicate that MDR may be a trainable response. A significant increase in the LF/HF percentage power was observed from the BA to AA and BA to AWC conditions and a shift from LF, sympathetic, parasympathetic activity to HF, primarily parasympathetic activity was observed across the protocols from BA to AWC.

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